Nordic Journal of Botany 28: 438–444, 2010
doi: 10.1111/j.1756-1051.2010.00658.x,

Subject Editor: Åsa Lankinen. Accepted 7 January 2010

Pollen source and resource limitation to fruit production in the rare species *Eremosparton songoricum* (Fabaceae)

Xiang Shi, Jian-Cheng Wang, Dao-Yuan Zhang, John Gaskin and Bo-Rong Pan

X. Shi (2000wwww@163.com), J.-C. Wang, D. Y. Zhang and B. R. Pan, Key Lab of Biogeography and Bioresource in arid land, Xinjiang Inst. of Ecology and Geography, Chinese Academy of Sciences, CN–830011 Urumqi, PR China. XS and JCW also at: Graduate Univ. of Chinese Academy of Sciences, CN–100049 Beijing, PR China. DYZ and BRP also at: Turpan Eremophytes Botanical Garden, Chinese Academy of Sciences, CN–838008 Turpan, PR China. – J. Gaskin, USDA Agricultural Research Service, Montana, 59270, USA.

Eremosparton songoricum (Litv.) Vass. is a rare, central Asian desert species which shows lower fruit set and seed set (< 16%) than most hermaphroditic species. We hypothesized that fruit production was limited by pollen and resources. To evaluate potential fruit abortion due to pollen limitation, supplemental hand-pollination was undertaken, the mating system was investigated and the foraging behavior of pollinators was recorded. To investigate possibile resource limitation, flowers and young pods were artificially removed and fertilization were manipulated. The results showed that under natural pollination, the number of pollen deposited on the stigma greatly exceed the number of ovules per ovary. Mating system experiments showed that the species is self-compatible, but depended on pollinators to set seeds. Supplemental outcross pollination increased fruit set significantly. The most frequent effective pollinator *Megachile terminate* Morawitz, was observed pollinating many flowers of the same individual plant (74.5 ± 1.3%). These results suggested that fruit production is affected by insufficient outcross pollen rather than by pollen quantity. Removal of 2/3 of the flowers and young pods led to significantly higher fruit set, as did addition of fertilizers (N–P–K: 0.025–0.05–0.013 g, N–P–K: 0.05–0.1–0.025 g) showing that reducing resource acceptors and increasing inorganic resources both helps to improve fruit set. We therefore conclude that reproductive success of *E. songoricum* is limited by both outcross pollen and available nutrients.

Angiosperms commonly mature fewer fruits and seeds than the number of flowers and ovules they produce (Stephenson 1981, Burd 1994), and the fruit set level is highly relevant to a plant's reproductive success (Guitian 1993). In the past three decades, a handful studies have examined the relative contributions of availability of resources, pollen transfer, flower or seed predation and genetic load to female reproduction in plants (Charlesworth 1989, Burd 1994, Huang and Guo 2002). Fruit set may be limited by pollen limitation and/or resource limitation (Bierzychudek 1981, Campbell and Halama 1993, Ashman et al. 2004). Pollen limitation in terms of inadequate pollen quantity or quality has been most commonly investigated, particularly in animal-pollinated plants (Burd 1994, Chris and Ruth 2002, Aizen and Harder 2007). There is also much evidence that fruit and (or) seed production is often limited by resources (Stephenson 1981, Campbell and Halama 1993, Brookes et al. 2008). However, these two factors are not mutually exclusive, as pollen limitation can occur in conjunction with resource limitation within a flowering season (Zimmerman and Pyke 1988, Ehrlén 1992, Dudash and Fenster 1996). Studies of causes and consequences of mortality from fertilization to seed and fruit maturation is important for understanding the reproductive strategies of plants (Carolina and Leonardo 1999), therefore, pollen limitation and resource limitation have achieved much attention in order to fully understand its ecological and evolutionary significance (Xiao et al. 2006).

Information about the reproduction of rare plants is of special importance for several reasons. First, reproduction may limit population growth, dispersal and colonization (Saunders and Sedonia 2006). Second, angiosperm reproduction often depends on interactions with animal pollinators, however, in scarce and fragmented populations plant–pollinator interactions may become disrupted and reproduction may be reduced as an effect of insufficient pollination (Moody-Weis and Heywood 2001). Therefore, it is necessary to propose some practical guidelines, based on knowledge of basic reproductive biology, to conserve the plants that are already at a risk of extinction (Falk and Holsinger 1991).

Eremosparton songoricum (Litv.) Vass. is a leafless perennial semi-shrub (up to 1 m in height) with hermaphrodite flowers (Zhang et al. 2008). Apart from sexual reproduction, *E. songoricum* spreads asexually through rhizomes. It is a rare inhabitant in central Asia, and it is the only species of the genus in China (Yin et al. 2006). *Eremosparton songoricum* is only found on the severely wind eroded mobile and semi-mobile sand dunes in the Gurbantunggut desert, Xinjiang, China (Zhang and Hai 2002) and on sand dunes around Lake Balkash in Kazakstan (Yin et al. 2006). It is a species with wind-resistance and sand-fixation characteristics, and it is widely used in fixing moving sand dunes and thus plays an important role in the maintenance of desert ecosystem stability. Severely disturbed by human activities, the populations are now fragmented and isolated. Moreover, the population size has been decreasing over the past decades, and gene flow among populations has become increasingly difficult (Zhang et al. 2008). Some weaknesses probably exist in the reproductive cycle, and human disturbance has added to the threat to this rare species.

An individual can produce 100–3000 flowers depending on plant size (Zhang et al. 2008). However, fruit set is lower (< 16%, Zhang et al. 2008) than in most self-compatible hermaphroditic species (e.g. 72%, Sutherland and Delph 1984). Similarily, seed set is lower (< 16%, Zhang et al. 2008) than in most hermaphroditic species (e.g. 50%–85%; Charlesworth 1989). Our objectives were to explore factors limiting reproductive success of this rare species to enhance potential conservation efforts. Two specific questions were: 1) is fruit production affected by pollen quantity and (or) quality? and 2) is fruit production affected by resource availability?

Material and methods

Study site and species

The study population was chosen in DuRe country; the most northern edge of Gurbantunggut desert (46°31′05″N, 88°33′04″E). The Gurbantunggut desert is the second largest desert in China with an area of 48 800 km^2, situated in the center of the Jungger Basin. Because of the 'blocking effect' of the Himalayan range, moist air currents from the Indian Ocean fail to reach the Gurbantunggut desert, resulting in a vast expanse of arid terrain. The sand-blowing wind in this area mostly occurs from April to June (Wang et al. 2003). The climate is typical for a temperate desert; the mean annual temperate is 5.0–5.7°C (with extreme temperatures exceeding −40°C and +40°C); the annual evaporation is 2000∼2800 mm, while the mean annual precipitation is 80–160 mm (Zhang and Chen 2002). The duration of flowering of *E. songoricum* varies from late May to late June and the fruiting period is from mid-July to late August. Based on flower morphology and anther dehiscence, the flowering process can be divided into four periods: pre-dehiscence, initial dehiscence, full dehiscence and withering (Ma et al. 2008) and the duration of stigma receptivity lasts from pre-dehiscence to the withering period. Our field investigation and lab experiments were conducted from 2004 to 2008.

Estimates of mating system

To examine the effect of pollen source on fruit-set levels, and the importance of pollinators on fruit set, we investigated the mating system of *E. songoricum*. We performed controlled hand-pollination experiments in 2007. For each experiment, three flowers growing in the same position were chosen from each of ten individuals, and we did the same operation in three different sand dunes within this population. Five experiments were designed as follows: 1) self-pollination, in which bagged flowers were hand pollinated with their own pollen; 2) geitonogamy, in which emasculated flowers were hand pollinated with pollen from the same individual; 3) outcrossing, in which emasculated flowers were pollinated with pollen from another plant, at least 20 m away from the recipient individual; 4) automatic self-pollination, in which buds were bagged throughout their flowering period without emasculation; 5) apomixis, in which buds were bagged after emasculation. All the emasculation treatments were conducted before anther dehiscence. Natural pollination was studied on 1453 flowers from ten randomly chosen plants. Fruit set was calculated as the percentage of fruits in relation to the total number of flowers produced, which was calculated at the expension phase (when the fruit is beginning expansion) and the mature phase (when the fruit is completely mature).

Estimates of pollen source effects and pollen quantity

To describe the effects of pollen quality on female reproductive success, we used supplemental hand pollination treatments in 2007. Each plant produces many hundreds of flowers in *E. songoricum*, and it is difficult to manipulate a whole plant in the field. In order to limit the effects of different-aged plants, we selected four flowering branches of approximately the same size, canopy position and exposure on each of six randomly chosen plants. Supplemental self-pollination, geitonogamy and outcross pollination was conducted (more than 20 m away from the pollinated one) on each one of the four flowering branches. We used the remaining branches as controls. All branches had 60 ∼ 80 flowers. All treatments were open to natural pollination. These supplemental pollination techniques were repeated daily from stigma emergence to flower senescence to ensure that the treatment coincided with stigma receptivity. Supplemental hand pollination was conducted between 9 June and 28 June. We recorded the fruit number and dissected the fruit to count the number of ovules. The undeveloped ovules were shrivelled and quantified at 40× magnification under a dissecting microscope. We then calculated the fruit set and seed set. Fruit set was calculated as the percentage of completely mature fruits in relation to the total number of flowers produced. Seed set was calculated as the percentage of seeds in relation to the total number of ovules per pod.

To determine whether pollen quantity was sufficient to fertilize all ovules, mature flowers (n = 30) in the peak of blooming were collected randomly and fixed immediately into FAA solution for measurement of stigmatic pollen load. Pistils were dissected carefully from flowers and immersed into 1% safranine solution, and pollen grains were then counted on the stigma under an optical microscope with 100× magnification. Each remaining ovary was carefully placed in 70% alcohol on a slide, and the number of ovules were counted at 40× magnification, and were recorded on a one-to-one correspondence of stigmatic pollen load.

Observation of the foraging behavior of pollinators

To find out the major mode of pollination, we followed the pollinators and recorded the foraging behavior in 2007. The captured insects were sampled for pollen types by touching fuchsin gel to the insect's body (Liu and Koptur 2003). The gel was then melted onto a slide for examination under an optical microscope with 100× magnification. Those pollinators which carried pollen of *E. songoricum* and were in contact with anthers and/or stigmas, resulting in pollination, were considered as effective pollinators in the wild population. Of the effective pollinators, at least five of each species were sampled and identified at Xinjiang Univ. We followed the most frequent effective pollinator and recorded the visits within- and between-plant individuals, and these observations were replicated 30 times.

Estimates of effects of available resources on reproductive success

To determine how resource availability affects female reproductive success, we manipulated experimental flowers by artificial flower removal, young pod removal and by adding nutrients. On each of the 20 randomly chosen individuals, we chose three flowering branches of approximately the same size and canopy position in 2007. Flowers and young pods were removed on ten of the 20 individuals respectively. 0, 1/3 and 2/3 of all flowers or young pods were removed on each of the three branches. Initially, all branches had 70–90 flowers. Every attempt was made to ensure that flowers and young pods were removed at the initial development and from the same position. The fruit set in mature phase was then calculated with respect to the number of flowers and young pods after removal. Seed set per pod was also calculated.

Three different concentrations of fertilizer (N–P–K = 2–4–1, N = 0.025–0.05–0.1 g) were added to the soil of 12 individuals, growing in similar habitat and with similar crown width. We replicated the supplemental treatment of inorganic resources every 15 days from 31 May (in the beginning of anthesis) to 15 July (fruit maturation). In each treatment, 100 ml of the fertilizer solution was applied around the root zone of each plant. In addition, 12 individuals received 100 ml water as control. The mature phase fruit set as well as seed set per pod were measured in late July.

Data analysis

The results were analyzed using SPSS 16.0 software, and the data were described by mean ± SE. One-way ANOVAs with a posterior Tukey-HSD test were performed to compare the variation of fruit set between mating system treatments in expension phase and mature phase, fruit (seed) set among supplemental pollination treatments and after artificial removal treatments, as well as after supplemental inorganic-resource treatments. A paired t-test was performed to compare the variation of pollen number on the stigma and ovule number in the ovary. Data normality was comprehensively considered by Kolmogorov–Smimov and Shapiro–Wilk Index. Levene's test was used for checking the homogeneity of variances.

Results

Mating system

The results from the mating system treatments are summarized in Table 1. Fruit abortion in *E. songoricum* was frequent, with nearly 70% of the fruits aborted between the post-zygotic stage and fruit maturation. When the flowers were emasculated, bagged and not pollinated, no fruits developed, showing that no apomixis occurred. Few fruits in the expension phase and zero in mature phase were present when flowers were not emasculated, bagged and not pollinated, indicating that spontaneous autogamy rarely happened, and that pollination success relied on pollinators. Moreover, some natural autogamous selfing might occur as a result of frequent windy conditions that cause flowers to move, thereby shaking pollen onto the stigma. For emasculated, bagging and self-pollination, geitonogamy and outcross pollination, the fruit set (expension phase) showed no significant difference, indicating that *E. songoricum* is self-compatible. However artificial outcrossing yielded more than twice the number of fruits, as compared to self-pollination and geitonogamy, which indicated that outcross pollen contributed more to reproductive success. In conclusion, *E. songoricum* exhibited a mixed mating system.

Only two visitors were recorded as effective pollinators. The most frequent pollinator was *Megachile terminate* Morawitz, accounting for 89.3% of all the visits. Another frequent pollinator was a species of the Stenogastridae

Table 1. Fruit set (mean ± SE) of *E. songoricum* compared by one-way ANOVA with a Tukey-HSD test within each treatment in different phases (lower case letters) and between each pollination treatment (upper case letters) in 2007. 'Expansion phase' is the time when the fruit is beginning expansion, and 'Mature phase' is the time when the fruit is completely mature; n is the number of total manipulated flowers in each treatment. *different lower case and upper case letters are significantly different at the 0.01-level.

Treatments		Natural pollination	Automatic selfing	Self-pollination	Geitonogamy	Outcross pollination	Apomixis
Results (%)	Expansion phase	$88.61 \pm 3.31^{a,\ A}$ (n = 1453)	$8.90 \pm 0.85^{a,\ B}$ (n = 90)	$89.99 \pm 1.20^{a,\ A}$ (n = 88)	$88.56 \pm 2.17^{a,\ A}$ (n = 89)	$88.55 \pm 2.52^{a,\ A}$ (n = 90)	$0^{a,\ C}$ (n = 90)
	Mature phase	$14.47 \pm 1.20^{b,\ B}$ (n = 1453)	$0.02 \pm 0.01^{a,\ C}$ (n = 90)	$12.93 \pm 1.28^{b,\ B}$ (n = 88)	$13.05 \pm 0.32^{b,\ B}$ (n = 89)	$34.10 \pm 1.31^{b,\ A}$ (n = 90)	$0^{a,\ C}$ (n = 90)

family which was not further identified. These two pollinators were in contact with anthers and (or) stigma with their body parts, where pollen was deposited during foraging. The 30 *M. terminate* individuals visited flowers from the same and different ramets, which accounted for 74.5 ± 1.3% and 24.3 ± 1.4% of the entire foraging activities, respectively. Therefore, geitonogamy is probably the major mode of pollination in *E. songoricum.*

Pollen limitation

A one-way ANOVA showed that there were significant differences between treatments ($F = 86.96$, $p = 0.0001$). Neither supplemental self-pollination ($p = 0.99$) nor geitonogamy ($p = 0.98$) had a significant effect on fruit set. Branches that received additional outcrossed pollen produced more fruits than controls did ($p = 0.0001$). None of the three treatments showed significant effects on seed set ($F = 0.46$, $p = 0.71$, Fig. 1).

The number of pollen on the stigmas (135.87 ± 8.62) were significantly higher than the number of ovules in the ovary (8.33 ± 0.19) ($t = 13.61$, $DF = 29$, $p < 0.001$); which indicated that the amount of pollen was sufficient to fertilize all ovules.

Resource limitation

The fruit set (with respect to the number of flowers and remaining young pods) were tested by one-way ANOVAs, and showed that there were significant differences between treatments in both the flower removal ($F = 4.59$, $p = 0.02$) and the young pod removal ($F = 16.90$, $p = 0.0001$) experiments. Neither removal of 1/3 of the flowers, nor removal of young pods had an effect on fruit set ($p_{flowers} = 0.53$, $p_{pods} = 0.24$), but removal of 2/3 of flowers and young pods led to a significantly higher fruit set ($p_{flowers} = 0.02$, $p_{pods} = 0.000$) than for controls (Fig. 2a–b). Neither flower removal ($F = 0.10$, $p = 0.91$) nor young pod removal ($F = 0.09$, $p = 0.91$) had a significant effect on seed set (Fig. 2c–d).

After artificial fertilization, we found that there were significant differences between treatments ($F = 18.12$, $p = 0.0001$). Compared with control, supplemental solutions of N–P–K = 0.05–0.1–0.025 g ($p = 0.0001$) and N–P–K = 0.025–0.05–0.013 g ($p = 0.003$) had significant effects on fruit set, while supplemental N–P–K: 0.1–0.2–0.05 g had no effect on fruit set ($p = 0.760$). None of the fertilzer solutions had an effect on seed set compared with the control ($F = 1.48$, $p = 0.23$, Fig. 3).

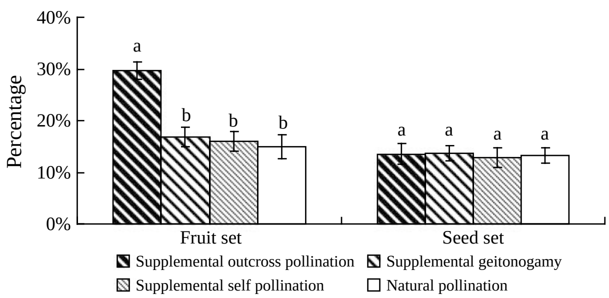

Figure 1. Comparison of fruit set in mature phase and seed set (mean ± SE) between natural pollination and supplementation treatments of *E. songoricum* by one-way ANOVA with a Tukey-HSD test. Different letters show a significant difference at the 0.01-level.

Discussion

Limitation of female reproductive success due to inadequate pollen receipt appears to be a common phenomenon in plants (Snow 1986, Dudash and Fenster 1996, Harder and Aizen 2007). The results of our study indicate that the number of pollen grains deposited on stigmas exceed the number of ovules per ovary. Snow (1986) reported that if the compatible pollen on the stigma was numerous enough to fertilize a sufficient number of ovules, the quantity of pollen would not affect fruit (seed) production. *Eremosparton songoricum* is a self-compatible species, and we therefore suggest that, at the level of a single flower, the number of pollen grains deposited on the stigma under natural pollination is not the main factor restricting fruit (seed) production.

Pollen quality is another frequent factor affecting seed production in nature (Waser and Price 1989, Ashman et al. 2004, Aizen and Harder 2007). Geitonogamy (self-pollination between flowers on the same plant, accomodated by bees) was 74.5 ± 1.3% in *E. songoricum*, and this major pollination mode would inevitably lead to a lack of outcrossed pollen. When combined with the results that the fruit set doubled after supplemental outcross pollination compared with the control, we concluded that fruit set is pollen limited, and this limitation is mainly due to pollen quality. Pollen limitation has been widely reported for entomophilous taxa in fragmented, isolated and sparse populations (Moody-Weis and Heywood 2001), and pollen limitation is even more serious in small and isolated populations (Lázaro and Traveset 2006). We therefore suggest that other fragmented populations that are smaller than the one studied here are more at risk when it comes to pollen limitation.

For self-compatible species, geitonogamy will lead to self-fertilization and may result in inbreeding depression (Young et al. 1996, Yang et al. 2005). In *E. songoricum*, there were no significant differences among artifical self-pollination, geitonogamy and outcross pollination with respect to fruit set (expension phase). However, pollination by self-pollen and geitonogamous pollen exclusively decreased the fruit set (mature phase) compared with that of artifical outcrossing. These results indicate that inbreeding depression plays a role during the period from fertilization to fruit maturation, as previously reported in another Fabaceae species (*Lupinus arboreus*) (Pamela and John 2000).

Although pollen limitation may be responsible for the low fruit production found in this study, fruit production may nevertheless be resource limited (Asikainen and Mutikainen 2005). Stephenson (1981) insisted that pollen limitation is one of the extrinsic reasons leading to fruit abortion, while resource limitation is the intrinsic reason. Removal of 2/3 of the flowers and young pods led to a significantly higher fruit set. This means that limited

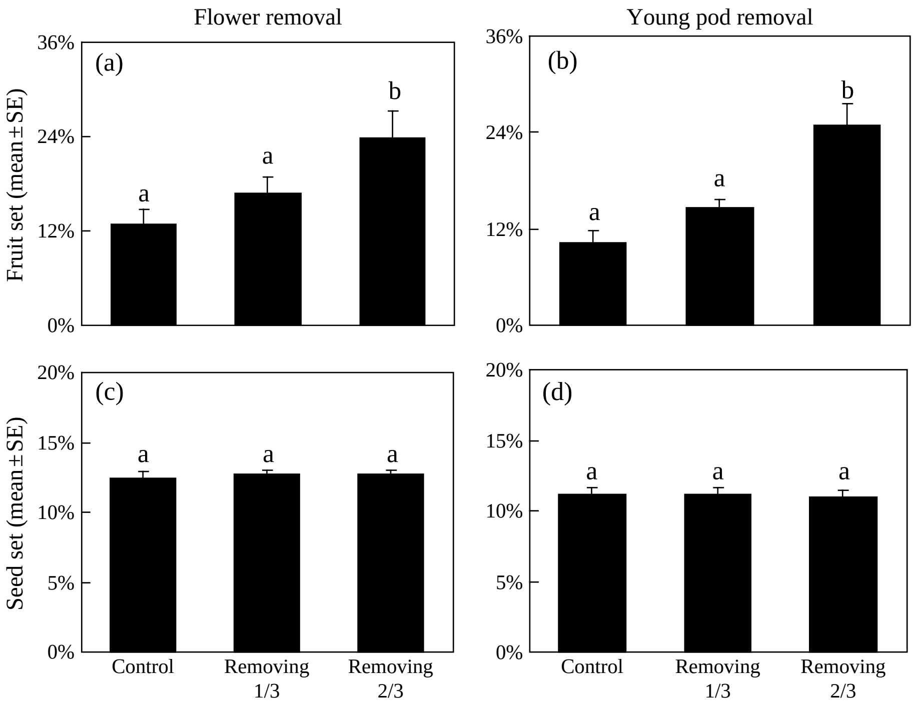

Figure 2. Comparison of fruit set in mature phase and seed set (mean+SE) between artificial removal and control of *E. songoricum* by one-way ANOVA with a Tukey-HSD test. Different letters show a significant difference at the 0.01-level.

resources will more effectively act on the offspring when removing most of the resource acceptors (Yang et al. 2005). Other studies have found that fruits that were initiated were also aborted, probably due to resource limitation (Stephenson 1981, Sutherland and Delph 1984, Medrano et al. 2000). In addition, fruit set increased 1.4 times after removing 2/3 of the young pods compared with 0.85 times after removing 2/3 of the flowers, which was remarkable. Dry weight per fruit was about two times that of a flower (flower: $n = 30$, 0.501 ± 0.004 mg; fruit: $n = 30$, 0.948 ± 0.004 mg, X. Shi and J. C. Wang unpubl.), indicating that a fruit is much more costly to produce than a flower, and that the resources required to produce one fruit is approximately equivalent to that of producing two flowers (Nobuhiko 2000). This result is in accordance with Ehrlén (1992), who argued that a low fruit-set level was related to high consumption when fruits developed. An addition of resources often leads to an increase in the proportion of

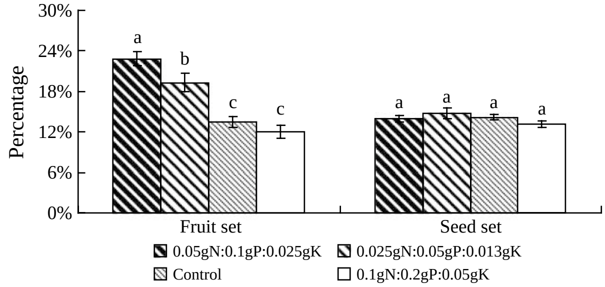

Figure 3. Comparison of fruit set and seed set (mean ± SE) after adding different concentrations of fertilizers and control of *E. songoricumin* by one-way ANOVA with a Tukey-HSD test. Different letters show a significant difference at the 0.01-level.

fruits that mature (Stephenson 1981, Yang et al. 2005, Brookes et al. 2008). The application of fertilizer solutions (N–P–K) during flowering and fruiting periods significantly decreased fruit abortion in *E. songoricum.* These results would suggest that in the harsh desert habitat, where mobile and semi-mobile sandy soil is predominant, mineral nutrients are a limiting resource (Zhang et al. 2008).

Somewhat surprisingly, seed set per pod did not change significantly after supplemental pollination, artificial removal of flowers or pods, or addition of fertilizers. Because there was a limitation in both pollen source and (or) available resources, this should lead to an increase in seed set after pollen supplementation, flower and pod removal as well as supplemental fertilization (Campbell and Halama 1993, Asikainen and Mutikainen 2005). The implications and causes of this phenomenon have not, to our knowledge, been discussed in any detail. From an evolutionary viewpoint, if the plant distributes its limited available resources equally to all of the ovules, each ovule might not get enough nutrients to yield a high quality seed (Charlesworth 1989). Our field investigation supported this argument; mean dry weight per seed from a pod which produced more than one seed (16.23 ± 0.30 mg) was significantly lower than a pod which only produced one seed (18.44 ± 0.32 mg) (X. Shi and J. C. Wang unpubl.). While not at the level of a single flower, but at the level of a flowering branch or individual, seed set did increase with an increase in fruit set. Salisbury (1942) and Johnson and Cook (1968) have proposed that, for plants, the optimal number of seeds is the maximum number which can be provided with adequate resources. To achieve the optimal number, plants must have the ability to control seed set, and one way to do this is through controlling the number of mature fruits per flower (Harper 1977, Primack 1978). In *E. songoricum*, producing one seed per pod may be the optimal choice, as the single seed is likely to be of higher quality. We therefore speculate that there may be physiological constraints which, in order to increase offspring fitness, tend to disperse the complementary resources into more fruits, rather than into more seeds per pod, to optimize reproductive effort.

The limitation of our present study is that only in a single year of *E. songoricum* was examined, and hence, the results cannot be used to draw general conclusions about the whole species over varying years. (The following year's investigation, in which we operated with the same method, faced serious seed predation when the fruit matured. According to our results from 2006–2008, we found that the pollinators visited flowers exclusively within individuals). However, many other studies of pollen and resource limitation in perennial species have been performed during one flowering season (Bierzychudek 1981, Holtsford 1985, Lawrence 1993, Lehtilä and Syrjänen 1995, Yang et al. 2005).

In conclusion, fruit abortion was frequent in *E. songoricum*. Limitations in outcross pollen and available resources appear to be reasonable explanations for this phenomenon. Pollen limitation can pose a threat to population persistence (Karkkainen et al. 1999). Chris and Ruth (2002) argued that the impact of pollen limitation will become apparent when a minimum threshold has been reached. According to the observations made during 2004–2009, one population (10×10 m, situated at 44°58′13N, 88°24′70E) had no pollinators and few fruits for the entire observation period. This population was first located in 2004, but had already disappeared five years later. With regards to these imporant findings, apiculture of effective pollinators should be beneficial to outcross pollination and protection of the existing population of the rare species *E. songoricum*.

Acknowledgements – The authors gratefully acknowledge the assistance and advice of Prof. Tan Dunyan from Xinjiang Agriculture Univ. Insect specimens were identified by Dr Hu Hongying at Xinjiang Univ. This work was supported by grants from National Science Foundation project of China (no. 30970547), Key Knowledge Innovation project of the Chinese Academy of Sciences (KSCX2-YW-Z-1020) and the National Basic Research Program, China (no. 2009CB825104).

References

Aizen, M. A. and Harder, L. D. 2007. Expanding the limits of the pollen-limitation concept: effects of pollen quantity and quality. – Ecology 88: 271–281.

Ashman, T. L. et al. 2004. Pollen limitation of plant reproduction: ecological and evolutionary causes and consequences. – Ecology 85: 2408–2421.

Asikainen, E. and Mutikainen, P. 2005. Pollen and resource limitation in a gynodioecious species. – Am. J. Bot. 92: 487–494.

Bierzychudek, P. 1981. Pollinator limitation of plant reproductive effort. – Am. Nat. 117: 838–840.

Brookes, R. H. et al. 2008. A test of simultaneous resource and pollen limitation in *Stylidium armeria*. – New Phytol. 179: 557–565.

Burd, M. 1994. Bateman principle and plant reproduction: the role of pollen limitation in fruit and seed set. – Bot. Rev. 60: 83–139.

Campbell, D. R. and Halama, K. L. 1993. Resource and pollen limitations to lifetime seed production in a natural plant population. – Ecology 74: 1043–1051.

Carolina, T. and Leonardo, G. 1999. Factors constraining fruit set in *Mandevilla pentlandiana* (Apocynaceae). – Bot. J. Linn. Soc. 129: 187–205.

Charlesworth, D. 1989. Evolution of low female fertility in plants pollen limitation, resource allocation and genetic load. – Trends Ecol. Evol. 4: 289–292.

Chris, W. and Ruth, N. Pollination failure in plants: why it happens and when it matters. – Trends Plant Sci. 7: 270–277.

Dudash, M. R. and Fenster, C. B. 1996. Multiyear study of pollen limitation and cost of reproduction in the iteroparous *Silene virginica*. – Ecology 78: 484–493.

Ehrlén, J. 1992. Proximate limits to seed production in a herbaceous perennial legume, *Lathyrus vernus*. – Ecology 73: 1820–1831.

Falk, D. A. and Holsinger, K. E. 1991. Genetics and conservation of rare plants. – Oxford Univ. Press.

Guitian, J. 1993. Why *Prunus mahaleb* (Rosaceae) produce more flowers than fruits. – Am. J. Bot. 80: 1305–1309.

Harper, J. L. 1977. The population biology of plants. – Academic Press.

Holtsford, T. P. 1985. Nonfruiting hermaphroditic flowers of *Calochortus leichtlinii* (Liliaceae): potential reproductive functions. – Am. J. Bot. 72: 1687–1694.

Huang, S. Q. and Guo, Y. H. 2002. The variation of pollination and resource limitation in a low seed-set tree, *Liriodendron chinense* (Magnoliaceae). – Bot. J. Linn. Soc. 140: 31–38.

Johnson, M. P. and Cook, S. A. 1968. Clutch size in buttercups. – Am. Nat. 102: 405–411.

Karkkainen, K. et al. 1999. Why do plants abort so many developing seeds: bad offspring or bad maternal genotypes? – Evol. Ecol. 13: 305–317.

Lawrence, W. S. 1993. Resource and pollen limitation: plant size-dependent reproductive patterns in *Physalis longifolia*. – Am. Nat. 141: 296–313.

Lázaro, A. and Traveset, A. 2006. Reproductive success of the endangered shrub *Buxus balearica* Lam. (Buxaceae): pollen limitation, and inbreeding and outbreeding depression. – Plant Syst. Evol. 261: 117–128.

Lehtilä, K. and Syrjänen, K. 1995. Positive effects of pollination on subsequent size, reproduction, and survival of *Primula veris*. – Ecology 76: 1084–1098.

Liu, H. and Koptur, S. 2003. Breeding system and pollination of a narrowly endemic herb of the lower florida keys: impacts of the urban-wildland interface. – Am. J. Bot. 90: 1180–1187.

Ma, W. B. et al. 2008. Flowering phenology and reproductive features of the rare plant *Eremosparton songoricum* in desert zone, Xinjiang, China. – J. Plant Ecol. 32: 760–767.

Medrano, M. et al. 2000. Patterns of fruit and seed set within inflorescences of *Pancratium maritimum* (Amaryllidaceae): nonuniform pollination, resource limitation, or architectural effects? – Am. J. Bot. 87: 493–501.

Moody-Weis, J. M. and Heywood, J. S. 2001. Pollination limitation to reproductive success in the Missouri evening primrose, *Oenothera macrocarpa* (Onagraceae). – Am. J. Bot. 88: 1615–1622.

Nobuhiko, S. 2000. Pollinator limitation and resource limitation of seed production in the Scotch broom, *Cytisus scoparius* (Leguminosae). – Plant Species Biol. 15: 187–193.

Pamela, M. K. and John, L. M. 2000. Outcrossing rate and inbreeding depression in the perennial yellow bush lupine, *Lupinus arboeus* (Fabaceae). – Am. J. Bot. 87: 652–660.

Primack, R. B. 1978. Regulation of seed yield in *Plantago*. – J. Ecol. 66: 835–847.
Salisbury, E. J. 1942. The reproductive capacity of plants. Studies in quantitative biology. – G. Bell.
Saunders, N. E. and Sedonia, D. S. 2006. Reproductive biology and pollination ecology of the rare Yellowstone park endemic *Abronia ammophila* (Nyctaginaceae). – Plant Species Biol. 21: 75–84.
Snow, A. A. 1986. Pollination dynamics in *Epilobium canum* (Onagraceae): consequences for gametophytic selection. – Am. J. Bot. 73: 139–151.
Stephenson, A. G. 1981. Flower and fruit abortion: proximate causes and ultimate functions. – Annu. Rev. Ecol. Syst. 12: 253–279.
Sutherland, S. and Delph, L. F. 1984. On the importance of male fitness in plants: patterns of fruit set. – Ecology 65: 1093–1104.
Wang, X. Q. et al. 2003. Stabilization of dune surface and formation of mobile belt at the top of longitudinal dunes in Gurbantunggut Desert, Xinjiang, China. – J. Desert Res. 23: 126–131.
Waser, N. M. and Price, M. V. 1989. Optimal outcrossing in *Ipomopsis aggregate*: seed set and offspring fitness. – Evolution 43: 1097–1109.
Xiao, Y. A. et al. 2006. Pollen and resource limitation to lifetime and seed production in a wild population of the endangered plant *Disanthus cercidifolius* Maxim. var. *longipes* H. T. Chang (Hamamelidaceae). – Acta Ecol. Sin. 26: 496–503.
Yang, C. F. et al. 2005. Resource limitation and pollen source (self and outcross) affecting seed production in two louseworts, *Pedicularis siphonantha* and *P. longiflora* (Orobanchaceae). – Bot. J. Linn. Soc. 147: 83–89.
Yin, L. K. et al. 2006. Rare and endangered higher plants endemic to Xinjiang Urumqi. – Xinjiang Sci. Techn. Publ. House.
Young, A. et al. 1996. The population genetic consequences of habitat fragmentation for plants. – Trends Ecol. Evol. 11: 413–418.
Zhang, D. Y. et al. 2008. Distribution and bio-ecological characteristics of *Eremosparton songoricum*, a rare plant in Gurbantunggut desert. – J. Desert Res. 28: 430–436.
Zhang, L. Y. and Chen, C. D. 2002. On the general characteristics of plant diversity of Gurbantunggut sandy desert. – Acta Ecol. Sin. 22: 1923–1932.
Zhang, L. Y. and Hai, Y. 2002. Plant communities excluded in the book of 'The vegetation and its utilization in Xinjiang'. I. The desert plant communities. – Arid Land Geogr. 25: 84–89.
Zimmerman, M. and Pyke, G. H. 1988. Reproduction in *Polemonium*: assessing the factors limiting seed set. – Am. Nat. 131: 723–738.